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Source: *Journal of Medical Entomology*, 37(2):216-221. 2000.

Published By: Entomological Society of America

DOI: <http://dx.doi.org/10.1603/0022-2585-37.2.216>

URL: <http://www.bioone.org/doi/full/10.1603/0022-2585-37.2.216>

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Temporal and Spatial Variation of Phlebotomine (Diptera: Psychodidae) Community Diversity in a Cutaneous Leishmaniasis Endemic Area of Costa Rica

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J. Med. Entomol. 37(2): 216-221 (2000)

ABSTRACT Phlebotomine sand fly species composition and diversity were studied in Acosta, an area of Costa Rica endemic for cutaneous leishmaniasis. Two CDC miniature light traps per house were set overnight in 72 houses distributed in 6 localities, once a month, during the rainy seasons of 1994 and 1995. Of the 22 species collected, the most common were *Lutzomyia serrana* (Damasceno & Arouck), *L. youngi* Feliciangelli & Murillo, *L. ylephiletor* (Fairchild & Hertig), *L. shannoni* (Dyar), *L. cruciata* (Coquillett), *L. gomezi* (Nitzulescu), *L. sanguinaria* (Fairchild & Hertig), and *Warileya rotundipennis* (Fairchild & Hertig). The Czekanowsky index and the Sorensen index as modified by Bray and Curtis to consider abundance were used to compare species composition and β -diversity. Comparisons were made among localities within years, between years, and localities each year. The data strongly indicated that phlebotomine species composition is more stable than diversity or abundance.

KEY WORDS *Lutzomyia*, species composition, diversity, Leishmaniasis, Costa Rica

PHLEBOTOMINE SAND FLIES are vectors of several types of leishmaniasis in Neotropical America (Young and Arias 1992). Cutaneous leishmaniasis, caused by *Leishmania panamensis* (Lainson & Shaw), is endemic in some rural areas of Costa Rica (Zeledón et al. 1985), where it is an important disease in humans (Hidalgo et al. 1987), with an annual incidence of 9 per 1,000 inhabitants (Rojas 1993).

Different sylvatic and domiciliary scenarios have been suggested for the transmission of cutaneous leishmaniasis (Zeledón 1992). The sylvatic cycle is better understood; transmission occurs when people clear forested areas for new agricultural or urban development. The suspected vectors for this cycle are *Lutzomyia ylephiletor* (Fairchild & Hertig) and *L. trapidoi* (Fairchild & Hertig), and *Le. panamensis* has been isolated from field-collected individuals of these species (Zeledón and Alfaro 1973). The reservoirs include several species of mammals including the rodent, *Heteromys desmarestianus* (Desmarest), and 2 edentata, *Choloepus hoffmanni* (Illiger) and *Bradypus griseus* (Illiger). This cycle also occurs in other countries of Central America (Zeledón 1992).

A new domestic epidemiological pattern for this disease has been described in Panamá and Costa Rica (Zeledón 1992). Deforestation has resulted in the vectors invading the peridomestic environment and adapting to new larval habitats, resting places and sources of blood. The statistics of the Department of Dermatology of the Ministry of Health in Costa Rica shows that from 1975 to the present, children in the 0- to 10-yr age group accounted for 40% of the total

number of cases in the country; this percentage increased to 48% during the last decade. Concurrently, sand flies have been trapped more frequently inside houses and collected resting in animal shelters in close proximity to houses (Zeledón et al. 1984, Fajardo 1991, Herrero et al. 1992), indicating that the epidemiology of this disease has changed.

The household environment presents a unique habitat to which sand flies are attracted by factors that differ from the sylvatic cycle. Some of these factors also have been identified as determinants for the incidence of cutaneous leishmaniasis. Risk factors associated with house environments have been studied in Acosta (Rojas 1993), a rural county located 45 km SW of San José, Costa Rica. Three factors associated with increased risk of infection include the following: (1) keeping domestic animals beneath houses, (2) having dogs, and (3) having pigs. All of these factors were related to potential sources of blood for the insects, accumulation of chemical attractants such as carbon dioxide, or increase in the number of breeding places as a result of animal activity. Presence of a cement floor was a protective factor.

In a previous case-control study of the risk factors associated with domiciliary transmission (Rojas 1993), *L. ylephiletor* abundance was 45% higher in case-houses than in control-houses and the same housing characteristics associated with the presence of disease were associated with the increased frequency of female *L. ylephiletor* in houses. This epidemiological evidence indicated that this sandfly may play an important role as a vector in the domestic environment.

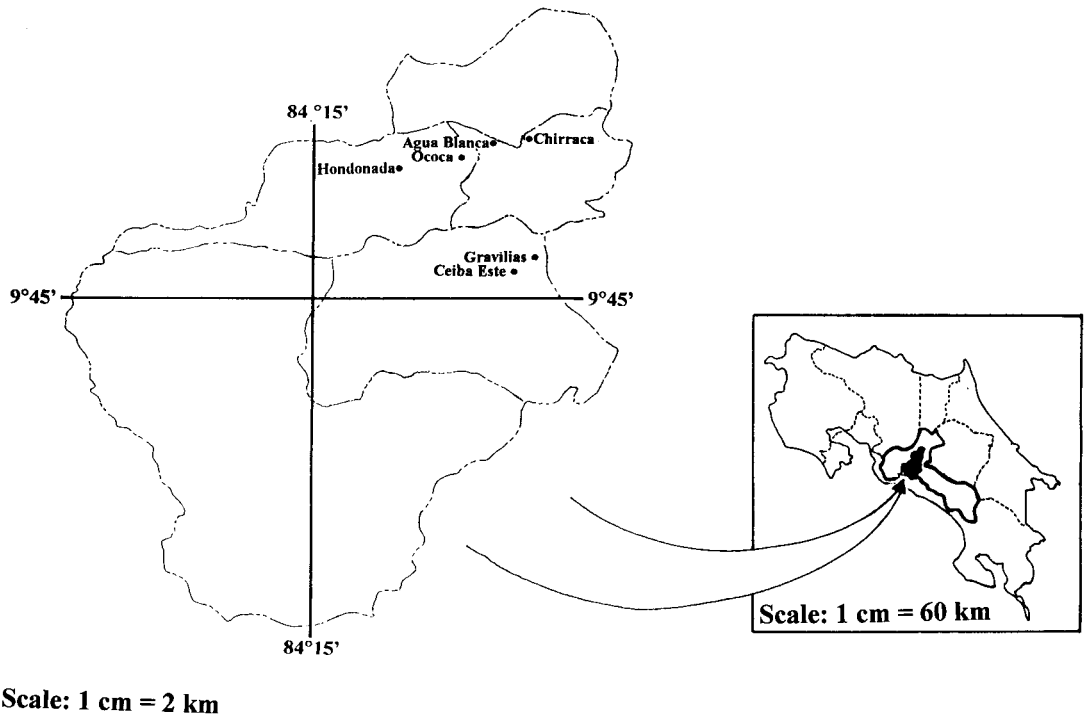


Fig. 1. Distribution of study localities in Acosta.

None of the other species of sand flies in the study area were associated statistically with cases (Rojas 1993).

Most reports on species composition and abundance of sand flies in cutaneous leishmaniasis endemic areas do not describe the relationships among the species sharing the same habitat. However, the presence of several species with similar feeding habits in the same habitat (household environment) indicates the development of complex interactions that only can be studied at the community level. The overall objective of the current study was to determine the sand fly species composition of the household environment. Our research tested the hypotheses that sand fly species attracted to houses are similar independently of the locality and that the main differences among localities are the result of changes in abundance that could be explained by environmental factors such as vegetative coverage within the populated area. A later paper will describe household infestation indices, endophilic indices, and the blood feeding preferences of female sand flies.

Materials and Methods

Study Area. The study was conducted in 6 rural localities in Acosta county, San José province, Costa Rica (latitude: 9° 48' 00" N; longitude: 84° 09' 50" W). We assumed that each locality was isolated, because the distance between any pair of localities was >1 km which is beyond the sand fly dispersal range (Alexander 1987, Young and Arias 1992, Alexander and

Young 1992) and because the intervening habitats were not suitable for sand flies. Data currently are not available concerning the species composition or diversity of sand flies in the extradomiciliary habitat.

Localities selected for study included Ococa, Ceiba Este, Chirracá, Hondonada, Agua Blanca, and Gravilias (Fig. 1). Human population sizes were 143, 336, 420, 105, 504, and 304 inhabitants, respectively. Localities are located from 1.6 to 6.0 km from the population main center (San Ignacio de Acosta). The 3 criteria used to select these study areas were as follows: (1) Cutaneous leishmaniasis transmission was indicated by new cases in children <10 yr old (Rojas 1993). During 1994 and 1995 the incidence rates were estimated to be 3.9 per 1,000 children at Agua Blanca, Gravilias and Ceiba Este and 3.1 per 1,000 children at Ococa, Hondonada and Chirracá. (2) Based on previous entomological sampling, similarity indices (Sorensen as modified by Bray and Curtis 1957) were known among localities (Herrero et al. 1993). (3) Localities differed in many ecological features such as the distribution of ornamental plants (*Balsamina impatiens*), coffee plantations, or ferns, and the distance of houses from forest patches.

Houses within localities were selected for study based on the presence of at least 1 risk factor for cutaneous leishmaniasis and included the following: constructed on stilts off the ground, presence of free-ranging pigs, and the presence of ≥1 dog as a pet. A total of 72 houses met the sampling criteria and these were distributed by locality as follows: 11 in Ococa, 11

Table 1. Abundance (corrected value × 1,000) of sand flies species in 6 localities of Acosta during 1994–1995

Species	Year	Locality					
		AB	GR	CE	OC	HO	CH
<i>L. serrana</i> ^a	1994	656	135	227	565	2305	793
	1995	901	433	604	1084	693	404
<i>L. youngi</i> ^a	1994	32	271	129	38	74	92
	1995	122	1388	369	287	87	274
<i>L. gomezi</i> ^a	1994	208	6	15	15	1148	16
	1995	139	94	45	130	614	55
<i>L. ylephiletor</i> ^a	1994	10	107	91	30	1583	38
	1995	46	174	63	72	307	20
<i>W. rotundipennis</i> ^a	1994	169	28	189	114	46	61
	1995	198	106	153	274	24	89
<i>L. cruciata</i> ^a	1994	40	17	32	29	278	61
	1995	12	58	8	17	100	7
<i>L. shannoni</i> ^a	1994	110	79	30	84	296	183
	1995	46	99	27	163	268	75
<i>L. sanguinaria</i> ^a	1994	26	17	15	15	46	8
	1995	46	35	9	46	55	14
<i>L. longipalpis</i> ^a	1994	299	28	15	8	46	—
	1995	192	40	63	26	24	—
<i>L. zeledoni</i>	1994	26	22	61	30	231	38
	1995	—	15	45	111	55	14
<i>L. b. mayuscula</i> ^a	1994	6	—	—	—	28	—
	1995	23	5	9	39	440	7
<i>L. hartmanni</i> ^a	1994	—	11	—	—	—	—
	1995	6	—	—	—	8	—
<i>L. bispinosa</i> ^a	1994	—	—	—	—	55	—
	1995	6	—	—	—	259	7
<i>L. vespertilionis</i>	1994	19	—	7	—	9	15
	1995	17	15	9	6	16	7
<i>L. gorbitzi</i>	1994	—	—	—	8	9	8
	1995	—	—	—	6	—	—
<i>L. runoides</i>	1994	6	—	—	8	9	—
	1995	29	—	—	13	31	—
<i>L. ovallesi</i> ^a	1994	6	6	7	—	92	—
	1995	6	—	9	—	8	—
<i>B. galindoi</i>	1994	—	—	—	—	111	—
	1995	—	—	—	—	—	—
<i>L. trinidadensis</i>	1994	—	—	—	—	37	—
	1995	—	—	—	—	—	—
<i>L. panamensis</i> ^a	1994	—	—	—	—	—	—
	1995	—	6	—	6	16	—
<i>L. rosabali</i>	1994	—	6	—	—	28	—
	1995	—	—	9	—	8	—
<i>L. atroclavata</i>	1994	—	—	—	—	46	—
	1995	—	—	—	—	31	—

Corrected value: number of individuals collected by species divided by collection effort, the number of houses × number of traps × number of nights × number of months.

^a Antropophilic species.

in Ceiba Este, 11 in Chirracá, 9 in Hondonada, 14 in Agua Blanca, and 16 in Gravilias.

Sampling Methods. During 1994–1995, entomological surveys were conducted during the rainy season

(May to November). CDC miniature light traps (Maroli et al. 1997) were set 1 m above the ground and operated from 1800 to 0600 hours. Two traps were set per house: one inside and the other outside. Trap locations were kept constant during the study period.

Table 2. Czekanowski Similarity Index in 6 localities of Acosta for 1994 and 1995

Locality	AB	GR	CE	OC	HO	CH
AB		0.78	0.83	0.80	0.88	0.77
GR	0.81		0.90	0.93	0.81	0.80
CE	0.85	0.88		0.83	0.85	0.80
OC	0.77	0.80	0.83		0.82	0.79
HO	0.70	0.73	0.75	0.75		0.73
CH	0.80	0.75	0.80	0.87	0.71	

1994: Lower left quadrant. 1995: Upper right quadrant. AB, Agua Blanca; GR, Gravilias; CE, Ceiba Este; OC, Ococa; HO, Hondonada; CH, Chirracá.

Table 3. Czekanowski Similarity Index in 6 localities of Acosta between 1994 and 1995

Locality	AB	GR	CE	OC	HO	CH
AB		0.90				
GR			0.77			
CE				0.92		
OC					0.89	
HO						0.87
CH						

AB, Agua Blanca; GR, Gravilias; CE, Ceiba Este; OC, Ococa; HO, Hondonada; CH, Chirracá.

Table 4. Beta Diversity Similarity Index of sand flies collected in 6 localities of Acosta for 1994 and 1995

Locality	AB	GR	CE	OC	HO	CH
AB		0.45	0.52	0.77	0.48	0.55
GR	0.39		0.52	0.51	0.40	0.54
CE	0.54	0.64		0.55	0.30	0.69
OC	0.69	0.43	0.60		0.50	0.59
HO	0.31	0.14	0.17	0.22		0.34
CH	0.66	0.43	0.53	0.76	0.30	

1994: Lower left quadrant. 1995: Upper right quadrant. AB, Agua Blanca; GR, Gravilias; CE, Ceiba Este; OC, Ococa; HO, Hondonada; CH, Chirraca.

Sand flies were collected in alcohol (70%). In the laboratory, sand flies were sorted by sex, cleared in phenol (80%), mounted in Hoyer's medium and identified to species using Young and Duncan (1994) and CIPA (1991).

Definition of variables and ecological analysis.

Abundance. To test the hypothesis that *L. serrana* was the most abundant species, a test for equality between 2 proportions from the same group with mutually exclusive categories was conducted (Microstat Ecosoft 1984). For each locality, the number of individuals per species was expressed as a proportion of the total sample collected and compared, 1×1 , using this test for proportions.

β -Diversity. To test the similarity, richness, and diversity among houses located in the 6 localities, 2 indices were selected:

The Czekanowski Similarity Index is calculated as $C_s = 2j / (a + b)$, where C_s is the index value, j is the number of species common to both localities and $a + b$ is the number of species in localities a and b . This index ranges from 1 when both habitats share exactly the same species to zero if there are no common species. It was used to compare species richness between localities and years and is independent of the number of individuals captured per species.

The Sorensen Similarity Index as modify by Bray and Curtis (1957) was calculated as $C_n = 2j_n / (a_n + b_n)$, where C_n is the Sorensen similarity index, j_n is the sum of the lower abundance values for the common species in localities a and b , and $a_n + b_n$ is the total number of individuals in communities a and b . This index will be equal to 1 when both habitats share exactly the same species and number of individuals per species and will equal 0 if there are no common spe-

cies. Sorensen similarity index was used to compare diversity between years and localities. This index combines richness and abundance and therefore is dependent on abundance (Southwood 1978).

Results

Abundance. Twenty-two species were captured (Table 1). *L. serrana*, *L. youngi*, *L. ylephiletor*, *L. shannoni*, *L. cruciata*, *L. gomezi*, *L. sanguinaria*, and *W. rotundipennis* occurred at all localities each year. *L. zeledoni*, *L. vespertilionis*, and *L. barretoii majuscula* were present in all localities on at least 1 occasion. *L. longipalpis* occurred at 5 of 6 localities in both years. Other species such as *L. trinidadensis*, *L. atroclavata*, and *B. galindoi* were rare and not captured in both years in the same locality. *L. serrana* was the most abundant species collected in all localities with the exception of Gravilias. The abundance of this species showed low variation between years (CV = 9%). In Gravilias, *L. youngi* was the dominant species.

β -Diversity. During 1994, the most similar communities using Czekanowski similarity index (CSI) were Ceiba Este and Gravilias (index = 0.88), whereas the most different were Hondonada and Agua Blanca (CSI = 0.70). During 1995, Gravilias and Ococa were most similar (CSI = 0.93), whereas Chirraca and Hondonada were most different (CSI = 0.73). Significant differences ($P < 0.05$) in species richness, between years (1994 versus 1995) for the same pair of localities was found between Agua Blanca and Hondonada (CSI₉₄ = 0.70 versus CSI₉₅ = 0.88) (Table 2). This index also compared species richness between years within each locality (Table 3), and ranged from 0.77 at Gravilias to 0.92 at Ceiba Este.

During 1994, the Sorensen similarity index (SSI) was highest for Chirraca and Ococa (SSI = 0.76) and lowest for Hondonada-Gravilias (SSI = 0.14). During 1995 the Agua Blanca and Ococa were most similar (SSI = 0.77). No significant differences ($P > 0.05$) were found in diversity between years for the same pair of localities (Table 4). The Sorensen similarity index also was used to compare diversity between years within each locality (Table 5), ranging from 0.41 at Hondonada to 0.76 at Agua Blanca.

Discussion

CDC miniature light traps were used to capture sand flies, using the household as our sampling unit. A previous study indicated that there were no differences between the sand fly fauna attracted to light and those collected resting during the day in this habitat (Herrero et al. 1992). Only 2 species, *L. panamensis* and *L. barretoii majuscula*, were not caught during our previous survey. Most of the sand fly species except for *L. rosabali* and *L. atroclavata* have been collected using light traps in other areas of Central America (Chaniotis et al. 1971, Murillo and Zeledón 1985, Fajardo 1991, Valle and Rivera 1995, CIPA 1997). However, it currently is unknown if these species are at-

Table 5. Beta Diversity Similarity Index of sand flies collected in 6 localities of Acosta between 1994 and 1995

Locality	AB	GR	CE	OC	HO	CH
AB	0.76					
GR		0.44				
CE			0.62			
OC				0.57		
HO					0.41	
CH						0.61

AB, Agua Blanca; GR, Gravilias; CE, Ceiba Este; OC, Ococa; HO, Hondonada; CH, Chirraca.

tracted equally to the white light of 6.3-V, 15 amp bulbs. Another limitation to our study is that the distance for attraction of sand flies to a CDC miniature light trap is unknown under tropical conditions during the rainy season.

Sand flies may be attracted to houses by blood meal hosts, resting habitats, or oviposition sites. This may be especially important in Acosta where the area has been deforested. Partitioning the habitat among species attracted to the domestic environment would be reflected in the number of individuals collected per species. Fourteen of the 20 species collected are known to feed on humans and 8 were among the most commonly captured. Potential vectors of *Leishmania panamensis* collected in this study included *L. panamensis*, *L. ylephiletor*, *L. shannoni*, *L. gomezi*, *L. ovallesi*, and *L. sanguinaria*; however, epidemiological data indicate that *L. ylephiletor* most probably is the vector for this parasite in Acosta.

The 6 communities showed annual variation in species richness and diversity. Such variations were affected more by changes in species abundance than by changes in species composition. *L. youngi*, *L. ylephiletor*, *L. cruciata*, and *W. rotundipennis* had the highest coefficient of variation between years (85, 68, 64, and 59%, respectively).

The Czekanowski similarity index was compared between years, and the coefficient of variation was <25%. A comparison of the species composition between localities showed that most pairs of localities were very similar between years. The range of similarity indexes between localities was 0.70–0.88 during 1994 and 0.73–0.93 during 1995. Similarly, the Czekanowski similarity index within each locality between years ranged from 0.77 to 0.92. These data indicate habitat continuity among sites and years. However, it was not possible to investigate the existence of a habitat *continuum* within the Acosta area, because sand flies were not sampled from extradomestic habitats. Further studies are required to sample these nondomestic habitats.

The Sorensen similarity index has been used by Herrero et al. (1993) and Alexander (1995) to analyze diversity of Phlebotomines in cutaneous leishmaniasis endemic areas. Variation between years was higher than for the Czekanowski similarity index, ranging from 0.14 to 0.79 during 1994 and from 0.33 to 0.77 during 1995. When compared between years within localities, the Sorensen similarity index ranged from 0.41 to 0.76. Previous results showed that diversity in Agua Blanca and Chirracá (SSI = 0.68) and Ceiba Este and Chirracá (SSI = 0.66) were very similar (Herrero et al. 1993).

The household as a sand fly environment needs further research to understand the reasons for domiciliation and in this area these type of studies would help to understand transmission.

Acknowledgments

We very much appreciate the help of the owners of the houses where this survey was conducted for their support

during these 2 yr. We thank Rodolfo Pereira and Heriberto Gutiérrez for their work in the field. This work was supported by the International Development Research Center (IDRC), Grant No. 92-0223-02.

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Received for publication 10 June 1998; accepted 23 August 1999.
